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Alstorisine A, a *nor*-monoterpenoid indole alkaloid from cecidogenous leaves of *Alstonia scholaris*

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ABSTRACT

Alstorisine A (**1**), a novel *nor*-monoterpenoid indole alkaloid with 6/5/6/6 fused-ring system, regarded as a key intermediate from melodinine E to mersicarpine, was isolated from the cecidogenous leaves of *Alstonia scholaris*. Its structure was identified on the basis of extensive spectroscopic analysis and the comparison of experimental and calculated ECDs. A plausible biogenesis was also postulated.

Plants defend against insect herbivory for their survival, while some insects spawn on the leaves instead of feeding them immediately so that their eggs can survive by sojourn in these leaves. Then, some special tissues more like tumor came into being, which were called galls and were induced by gall-formers such as insects and fungi on host plants. The chemical constituents of the cecidogenous plants may have some difference with the normal ones.[1] During the years of observation on *Alstonia scholaris*, we found that the leaves of *A. scholaris* may suffer galls on surface.[2] We wondered whether the constituents would have some changes due to the galls on the leaves. This inspired us to compare the difference between normal leaves and cecidogenous leaves. The HPLC fingerprint analysis of the extracts of two forms showed some obvious difference, that is, the contents of the main components changed and some trace compositions were produced (see Fig. 1 and Supplementary data). More importantly, cecidogenous leaves of *A. scholaris* seem not to have been studied chemically before. Motivated by the interesting phenomenon, intriguing architectures,[3,7] and bioactivities[4] of monoterpenoid indole alkaloids in this plant, a phytochemical study of the cecidogenous leaves was carried out. As a result, a *nor*-monoterpenoid indole alkaloid, alstorisine A (**1**) with a 6/5/6/6 fused-ring system was isolated. Furthermore, it was not distributed in healthy leaves, but appeared equally both in galls and the normal part of cecidogenous leaves at a concentration of 3 μg/g, which was indicated by LC–MS quantitative analysis (see Supplementary data). In alstorisine A, a carbon (C-5) of the skeleton was degraded compared to melodinine E, scholarisine G, and leuconoxine, which have attracted much attention for their total synthesis and asymmetric cascade catalysis.[5] Zhu and coworkers developed a unified strategy for the enantioselective synthesis of leuconolam-leuconoxine-mersicarpine subfamily of Aspidosperma alkaloids, while melodinine E served as springboard to reach leuconoxine, leuconodine A, leuconodine C, leuconodine F, and leuconolam.[5g] Then, alstorisine A (**1**) could be regarded as a key intermediate from melodinine E to mersicarpine.[5g] Besides, 20 known compounds were also isolated and identified as 4-aminobenzylamine, 3-(aminomethyl)-benzoic acid, (*Z*)-16-formyl-5α-methoxylstrictamine,[6] 5α-methoxylstrictamine,[7] picralinal,[8] picrinine,[8] (+)-geissoschizine,[9] (+)-vincadifformine,[10] strictamine,[11] vallesiachotamine,[12] isovallesiachotamine,[12] vallesamine,[13] akuammidine,[14] tubotaiwine,[15] tubotaiwine *N*-oxide,[16] burnamine,[17] scholarine,[18] (19*R*)-scholaricine,[19] (19*S*)-scholaricine,[19] and hedyotisol-A.[20] The new compound was tested for its bioactivity of regulating hippocampal neural stem cells (NSCs) proliferation in vitro. Reported herein is the isolation, structural elucidation, and proposed biogenic pathway of **1**.

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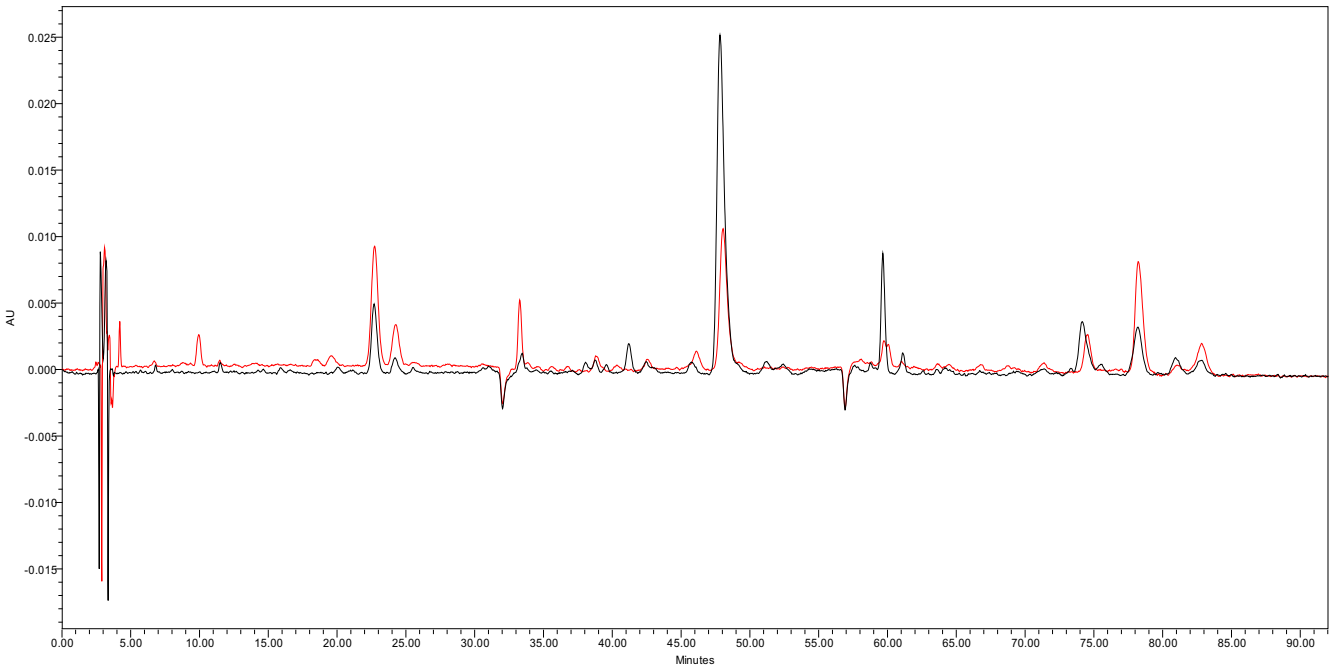


Figure 1. Fingerprint of the tested samples (black: total alkaloids from the cecidogenous leaves of *A. scholaris*; red: total alkaloids from the normal leaves of *A. scholaris*).

Air-dried and powdered leaves with galls of *A. scholaris* (10 kg) were extracted three times with MeOH under reflux conditions and the solvent was evaporated in vacuo. The extract was dissolved in 0.3% HCl, and the solution was filtrated and subsequently basified to pH 9–10, using ammonia. The solution was partitioned with EtOAc, affording aqueous and EtOAc phases. The EtOAc fraction (113 g) was chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH [from 30:1 to 0:1], to afford seven fractions (I–VII). Fraction II (18.5 g) was subjected to a preparative reversed phase C_{18}-MPLC column with a gradient flow of 30–80% (v/v) aqueous MeOH to yield six subfractions II-1–II-6. Subfraction II-5 (2.1 g) was further separated by silica gel to get the subfraction containing the target molecule. Compound **1** (20 mg) was purified from this subfraction via Prep-TLC using $CHCl_3$–MeOH (15:1) as an eluent.

Compound **1**[21] (Fig. 2) was isolated as a white amorphous powder and gave a positive reaction with Dragendorff's reagent. Its molecular formula was obtained as $C_{18}H_{22}N_2O_2$ by HRESIMS (*m*/*z* 299.1756 $[M+H]^+$) in association with ^1H and ^{13}C NMR data, which indicated 9 degrees of unsaturation. Its IR spectrum showed characteristic absorption bands at 3432 cm^{-1} for hydroxyl group, 1631 cm^{-1} for lactam group, and 1474 and 1099 cm^{-1} for an aromatic ring. The UV spectrum showed absorption maxima characteristic of β-anilinoacrylate chromophore (337, 264, 245, and 210 nm).[22] The ^1H NMR spectrum revealed the existence of a 1,2,4-trisubstituted benzene ring [δ_H 6.92 (1H, d, J = 2.3 Hz, H-9), 6.80 (1H, dd, J = 8.7, 2.3 Hz, H-11), 8.14 (1H, d, J = 8.7 Hz, H-12)]. The correlations of δ_H 8.14 (d, J = 8.7 Hz, H-12) with 6.80 (dd, J = 8.7, 2.3 Hz, H-11) in the ^1H–^1H COSY spectrum, as well as the correlations of δ_H 6.92 (d, J = 2.3 Hz, H-9) with δ_C 142.2 (C-7) in the HMBC spectrum, suggested that C-10 (δ_C 153.6) was substituted by a –OH function. The ^{13}C NMR (BB and DEPT) spectra of **1** displayed a total of 18 carbon resonances which were assigned to one methyl (δ_C 7.2), seven methylenes (δ_C 105.8, 39.3, 30.7, 29.3, 27.1, 23.9, and 20.2), three methines (δ_C 119.2, 116.9, and 107.1), and seven quaternary carbons (δ_C 169.2, 153.6, 142.2, 135.5, 128.8, 84.8, and 37.2) (Table 1). The spectral data as well as a series of monoterpenoid indole alkaloids (MIAs) isolated from *A. scholaris* suggested that **1** might be a MIA derivative. Unlike the other intact MIAs, in the HMBC spectrum of compound **1**, the correlations from δ_H 5.69 (s, H_a-6) and 5.29 (s, H_b-6) to δ_C 128.8 (C-8),

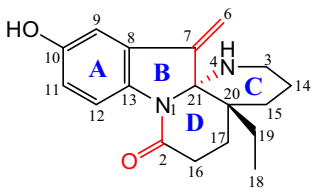


Figure 2. Structure of alstorisine A (**1**).

Table 1
^1H (600 MHz), ^{13}C (150 MHz) NMR, and HMBC data of **1** (δ in ppm) in $CDCl_3$

No.	^1H, mult. (J Hz)	^{13}C, type	HMBC
2		169.2, s	
3	3.11, td (12.1, 3.3) 2.75, overlap	39.3, t	15, 21
6	5.69, s 5.29, s	105.8, t	7, 8, 21
7		142.2, s	
8		128.8, s	
9	6.92, d (2.3)	107.1, d	7, 10, 11, 13
10		153.6, s	
11	6.80, dd (8.7, 2.3)	116.9, d	9, 10, 13
12	8.14 d (8.7)	119.2, d	8, 10, 13
13		135.5, s	
14	1.87, overlap 1.55, m	20.2, t	20
15	1.86, overlap 1.74, m	30.7, t	3, 14, 17, 19, 20, 21
16	2.74, overlap 2.46, m	29.3, t	2, 17, 20
17	2.75, overlap 1.48, m	23.9, t	2, 15, 19, 20, 21
18	0.79, t (7.5)	7.2, q	19, 20
19	1.44, q (7.5) 1.37, q (7.5)	27.1, t	15, 17, 18, 20, 21
20		37.2, s	
21		84.8, s	

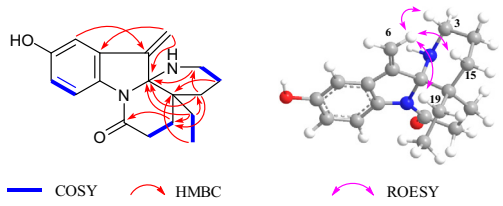


Figure 3. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of **1**.

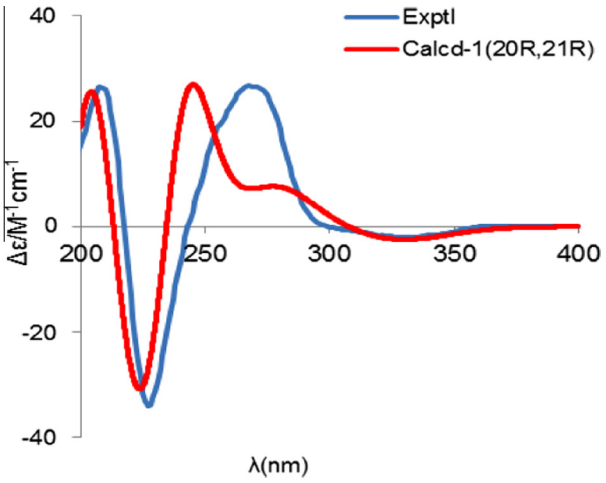
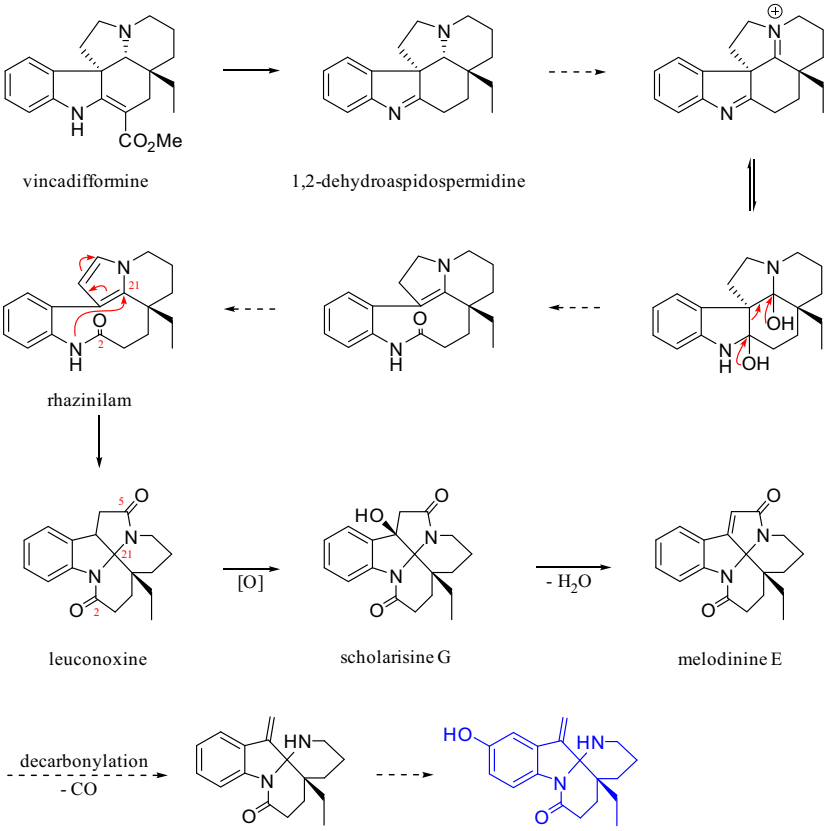


Figure 4. Calculated and experimental ECD spectra of **1** (red, calculated at the B3LYP-PCM/6-31G(d,p)//B3LYP/6-31G(d,p) level in MeOH; blue, experimental in MeOH).

142.2 (C-7), and 84.8 (C-21) suggested an uncommon exocyclic sp^2 methylene (δ_C 105.8, C-6) attached to C-7 of the indole ring directly.

Correlations of δ_H 3.11 (td, J = 12.1, 3.3 Hz, H_a-3) with 1.87 (overlap, H_a-14) and 1.55 (m, H_b-14) in the ^{1}H–^{1}H COSY spectrum, as well as the correlations of δ_H 1.86 (overlap, H_a-15) and 1.74 (m, H_b-15) with δ_C 39.3 (C-3); δ_H 1.87 (H_a-14) and 1.55 (H_b-14) with δ_C 37.2 (C-20) in the HMBC spectrum, suggested the linkage of C-20/C-15/C-14/C-3. Correlations of δ_H 3.11 (H_a-3) and 2.75 (H_b-3) with δ_C 84.8 (C-21) in the HMBC spectrum, established the linkage of C-3/N-4/C-21, which together with the established linkage of C-20/C-15/C-14/C-3 constructed a six-membered ring-C. The correlations of δ_H 0.79 (t, J = 7.5 Hz, H-18) with 1.44 (q, J = 7.5 Hz, H_a-19) and 1.37 (q, J = 7.5 Hz, H_b-19) in the ^{1}H–^{1}H COSY spectrum, as well as the correlations of δ_H 0.79 (H-18) with δ_C 37.2 (C-20) in the HMBC spectrum, indicated the direct connection between C-19 and C-20. Furthermore, in the HMBC spectrum, the correlations from δ_H 1.44 (H_a-19) and 1.37 (H_b-19) to δ_C 23.8 (C-17), 30.7 (C-15) and 84.8 (C-21), suggested the linkage of C-20/C-17, C-20/C-15, and C-20/C-21. Correlations of δ_H 1.48 (m, H_b-17)/2.46 (m, H_b-16) in the ^{1}H–^{1}H COSY spectrum, as well as the correlations of δ_H 2.75 (H_a-17), 1.48 (H_b-17) with a lactam carbonyl δ_C 169.2 (C-2), suggested the linkage of C-17/C-16/C-2/N-1, which together with the established linkage of C-21/C-20/C-17 constructed the six-membered ring-D. The deduction could explain the downfield chemical shift of C-21 (δ_C 84.8) in the ^{13}C NMR spectrum, for C-21 connecting two nitrogen atoms, which also met its degrees of unsaturation. Thus, the planar structure of **1** was elucidated to possess a 6/5/6/6 ring-fused system.

The relative configuration of **1** was established by ROESY spectrum (Fig. 3). In a molecular model, δ_H 5.69 (H_a-6) showed correlations with δ_H 3.11 (H_a-3), 1.86 (H_a-15), and 1.44, 1.37 (2H, H-19),



Scheme 1. Proposed biosynthetic pathway of **1**.

which placed the single bonds of C-21/N-4 and C-20/C-15 at the same side of ring-D to form the six-membered ring-C with chair configuration. Moreover, the absolute configuration of **1** was determined by comparing its experimental electronic circular dichroism (ECD) spectrum with that determined by time dependent density functional theory (TDDFT) calculations. The theoretical calculations of ECD were performed at B3LYP/6-31G (d,p) level in MeOH with PCM model on B3LYP/6-31G (d,p) optimized geometries in Gaussian 09 program.[23] The ECD spectrum generated for 20*R*,21*R* showing the positive (210, 264 nm) and negative (245 nm) Cotton effects (CE) were in good agreement with the experimental data of **1** (Fig. 4). Thus, the structure of alstorisine A was proposed finally as **1**.

The plausible biosynthesis of **1** could be traced back to a known compound vincadifformine via rhazinilam, and leuconoxine.[24] The oxidation of leuconoxine followed by dehydration led to form melodinine E which possessed a double bond at 6/7. Then decarbonylation and substitution of melodinine E yield alstorisine A (**1**) (Scheme 1). Alstorisine A, the first example of *nor*-leuconoxine alkaloids in the rhazinilam-leuconolam group of alkaloids, whose ring was cleaved, C-5 was degraded, and chiral carbons of C-20 and 21 were kept during biosynthesis.

Compound **1** was evaluated for its bioactivity of regulating hippocampal neural stem cells (NSCs) proliferation in vitro, but did not show significant activity in our bioassay.

Acknowledgments

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Supplementary data

Supplementary data (NMR, MS, CD, and HPLC fingerprint analysis spectra and computational methods for configuration determination of alstorisine A (**1**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.03.022.

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21. Alstorisine A (**1**), amorphous powder; $[\alpha]_D^{25}$ +86.7 (c = 0.1, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 210 (4.30), 245 (4.37), 264 (4.31), 337 (3.72) nm; IR (KBr) ν_{max} 3440, 3432, 2944, 1632, 1474, 1406, 1212, 1099 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; positive ESIMS m/z 299 $[M+H]^+$, 321 $[M+Na]^+$; positive HRESIMS m/z 299.1756 (calcd for $C_{18}H_{23}N_2O_2$ $[M+H]^+$, 299.1760).
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